Relationships of Human Papillomavirus Type, Qualitative Viral Load, and Age with Cytologic Abnormality

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Abstract

Persistent cervical infections with carcinogenic human papillomaviruses (HPV) cause virtually all cervical cancer. Cytologic abnormalities are the manifestations of HPV infections used to identify women at risk. To compare the potential of the full range of anogenital HPV genotypes to induce cytopathic effects, we examined the influences of HPV type, viral load, and age on cytopathology among 1,222 women having a single HPV type at enrollment into a 10,000-woman population-based study in Costa Rica. Cervical specimens were tested for ~40 HPV types by MY09/MY11 L1 primer PCR and type-specific dot blot hybridization. Types were organized by phylogenetic species and cancer risk. PCR signal strength served as a qualitative surrogate for viral load. Overall, 24.8% [95% confidence interval (95% CI), 22.4-27.3] of single prevalent HPV infections had concurrent abnormalities (atypical squamous cells or worse) ranging from 0.0% to 80.0% based on HPV type. Noncarcinogenic α3/α15 types, although highly prevalent, uncommonly caused cytologic abnormalities (13.1%; 95% CI, 9.8-17.0). In contrast, one quarter to nearly one half of infections with a single major carcinogenic species type (α9/α11/α7/α5/α6) produced abnormalities. Greater abnormalities were observed with increasing qualitative viral load of carcinogenic types; fewer abnormalities were observed among older women (>54 years). A high percentage (46.2%) of detected abnormalities in women infected with HPV16 or related α9 types were high grade or worse, consistent with strong carcinogenicity, compared with 10.7% in women infected with α7 types, including HPV18, a major cause of adenocarcinoma. The lack of evident severe abnormalities associated with HPV18 and related HPV types might have implications for screening for poorly detected glandular and α7-related lesions. (Cancer Res 2006; 66(20): 10112-9)

Introduction

More than 40 human papillomavirus (HPV) types infect the cervix. Most infections, including those by approximately 13 to 15 carcinogenic types, are transient. Persistent cervical infections by carcinogenic HPV types cause virtually all cervical cancer worldwide (1–3). HPV infection can lead to equivocal cytomorphologic changes referred to as atypical squamous cells (ASC), definite cytologic signs of HPV infection termed low-grade squamous intraepithelial lesions (LSIL), or cytologic signs of a potential cancer precursor designated as high-grade squamous intraepithelial lesions (HSIL; ref. 4). HSIL is the best cytologic correlate of histologic diagnoses of cervical intraepithelial neoplasia (CIN) grade 2, grade 3, or carcinoma *in situ*. Although these cytopathic manifestations of cervical HPV infections are used in Papanicolaou testing to identify women at risk for cervical cancer, the potential of individual HPV types to induce cytologic abnormalities has not been fully studied.

Numerous studies have attempted to determine whether HPV infection and high concentration of HPV DNA (HPV viral load) in cytologic specimens are predictors of detectable cytologic abnormalities and/or underlying histologic CIN (5–13). Many studies have relied on convenience populations rather than true population samples to evaluate these relationships. In addition, most studies have been restricted to HPV16 or carcinogenic types as a group. The results remain controversial and even incomplete for the less frequent, individual carcinogenic and noncarcinogenic types. Consideration of age adds another layer of complexity because the relationship between HPV infection with specific types and the likelihood of detecting cytologic abnormalities at different ages has not been fully characterized. Some previous cross-sectional analyses have suggested that HPV DNA prevalence and cytologic abnormality drop steadily and in parallel with age (14, 15). In comparison, other prevalence studies have revealed U-shaped age-specific HPV DNA prevalence curves for virtually every type, with higher prevalences in the younger and older women than in the middle-aged women (16–21). We therefore comprehensively examined the interrelationships of the full range of carcinogenic and noncarcinogenic HPV types, qualitative viral load, and age with cytologic abnormalities within a population-based cohort of ~10,000 randomly chosen women in Guanacaste, Costa Rica.

Materials and Methods

Study population. This population-based cohort study included participants from Guanacaste, Costa Rica enrolled between June 1993 and December 1994 with the approval of the National Cancer Institute (NCI) and Costa Rican institutional review boards (17, 22). Of the 11,742 potentially eligible subjects, 10,049 women provided written informed consent. Detailed methods of cohort recruitment, screening, and follow-up have been previously published (23).

After excluding women who were hysterectomized (n = 630), were virgins (n = 583), or refused a pelvic exam (n = 291), a baseline analytic group of 8,545 women was defined. After further excluding women who at enrollment were missing liquid-based cytology results (n = 469), had multiple infections (n = 658), had missing PCR results (n = 24), or were found to be positive only for a combination of rare HPV types (dot blot mix; n = 8) or uncharacterized

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types (n = 239), final analyses groups of 1,222 women with single HPV infections and 5,925 PCR-negative women were examined. Of the 469 women with missing cytology results, 15.8% (n = 74) had single HPV infections similar to those women with cytology results (15.1%; P = 0.7). Women positive for the rare HPV types in aggregate or for uncharacterized types were removed as we could not be certain that these women had single HPV infections. Multiple HPV infections were removed from the analysis because it was unclear to which type the cytologic abnormalities should be attributed. Of the 658 women with multiple infections, there were 75 (11.4%) women with ASC, 133 (20.2%) women with LSIL, and 58 (8.8%) women with HSIL or worse (37 women with HSIL-CIN2, 17 with HSIL-CIN3, 3 with cytologic interpretations of microinvasive cancer, and 1 with a cytologic interpretation

Table 1. Percentage of women with single HPV infections having any cytologic abnormalities

HPV type	Total N	% Abnormal (95% CI)*	Abnormal N	% ASC †	% LSIL	% HSIL+
All single infections ‡	1,222	24.8 (22.4-27.3)	303	44.2	32.0	23.8
α1/α8/α10	50	18.0 (8.6-31.4)	9	55.6	44.4	0.0
HPV6	23	17.4 (5.0-38.8)	4	25.0	75.0	0.0
HPV11	5	20.0 (0.5-71.6)	1	100.0	0.0	0.0
HPV74	1	100.0	1	0.0	100.0	0.0
HPV55	4	25.0 (0.6-80.6)	1	100.0	0.0	0.0
HPV40	4	50.0 (6.8-93.2)	2	100.0	0.0	0.0
HPV32	10	0.0	0			
HPV42	3	0.0	0			
α13	22	18.2 (5.2-40.3)	4	100.0	0.0	0.0
HPV54	22	18.2 (5.2-40.3)	4	100.0	0.0	0.0
α9	351	33.9 (29.0-39.1)*	119	28.6	25.2	46.2
HPV52 §	39	18.0 (7.5-33.5)	7	42.9	42.9	14.3
HPV67	5	80.0 (28.4-99.5)*	4	75.0	25.0	0.0
HPV33	23	30.4 (13.2-52.9)	7	71.4	14.3	14.3
HPV58	70	32.9 (22.1-45.1)	23	30.4	34.8	34.8
HPV16	156	38.5 (30.8-46.6)*	60	21.7	18.3	60.0
HPV31	46	26.1 (14.3-41.1)	12	0.0	41.7	58.3
HPV35	12	50.0 (21.1-78.9)*	6	50.0	16.7	33.3
α11	11	45.5 (16.7-76.6)	5	40.0	60.0	0.0
HPV73 ‖	9	55.6 (21.2-86.3)*	5	40.0	60.0	0.0
HPV34	1	0.0	0			
HPV64	1	0.0	0			
α7	218	25.7 (20.0-32.0)	56	51.8	37.5	10.7
HPV59	9	44.4 (13.7-78.8)	4	50.0	25.0	25.0
HPV18	39	30.8 (17.0-47.6)	12	58.3	25.0	16.7
HPV45	16	12.5 (1.6-38.3)	2	100.0	0.0	0.0
HPV70	85	22.4 (14.0-32.7)	19	63.2	36.8	0.0
HPV39	30	43.3 (25.5-62.6)*	13	23.1	69.2	7.7
HPV68	13	38.5 (13.9-68.4)	5	60.0	20.0	20.0
HPV85	26	3.8 (0.1-19.6)*	1	0.0	0.0	100.0
α5	84	31.0 (21.3-42.0)	26	53.9	26.9	19.2
HPV26 ‖	5	20.0 (0.5-71.6)	1	0.0	0.0	100.0
HPV51	72	33.3 (22.7-45.4)	24	54.2	29.2	16.7
HPV82 ‖	6	16.7 (0.4-64.1)	1	100.0	0.0	0.0
HPV69	1	0.0	0			
α6	119	30.3 (22.2-39.3)	36	36.1	55.6	8.3
HPV53	74	20.3 (11.8-31.2)	15	53.3	26.7	20.0
HPV56	27	48.1 (28.7-68.1)*	13	15.4	84.6	0.0
HPV66	18	44.4 (21.5-69.2)	8	37.5	62.5	0.0
α15	118	13.6 (8.0-21.1)*	16	75.0	18.8	6.3
HPV71	118	13.6 (8.0-21.1)*	16	75.0	18.8	6.3
α3	249	12.9 (9.0-17.7)*	32	65.6	28.1	6.3
HPV61	101	5.0 (1.6-11.2)*	5	60.0	20.0	20.0
HPV72	10	10.0 (0.3-44.5)	1	100.0	0.0	0.0
HPV62	54	16.7 (7.9-29.3)	9	88.9	11.1	0.0
HPV81	26	23.1 (9.0-43.6)	6	66.7	33.3	0.0
HPV83	38	13.2 (4.4-28.1)	5	60.0	20.0	20.0
HPV89	4	25.0 (0.6-80.6)	1	0.0	100.0	0.0
HPV84	16	31.3 (11.0-58.7)	5	40.0	60.0	0.0

(*Continued on the following page*)

Table 1. Percentage of women with single HPV infections having any cytologic abnormalities (Cont'd)

HPV type	Total N	% Abnormal (95% CI)*	Abnormal N	% ASC †	% LSIL	% HSIL+
α1/α8/α10/α13	72	18.1 (10.0-28.9)	13	69.2	30.8	0.0
α9/α11	362	34.3 (29.4-39.4)*	124	29.0	26.6	44.4
α9/α11(HPV16-)	206	31.1 (24.8-37.9)*	64	35.9	34.4	29.7
α7	218	25.7 (20.0-32.0)	56	51.8	37.5	10.7
α5/α6	203	30.5 (24.3-37.4)*	62	43.6	43.6	12.9
α3/α15	367	13.1 (9.8-17.0)*	48	68.8	25.0	6.3
All carcinogenic	570	34.4 (30.5-38.4)*	196	33.7	33.7	32.7
Carcinogenic (HPV16−)	414	32.9 (28.3-37.6)*	136	39.0	40.4	20.6
Noncarcinogenic	652	16.4 (13.6-19.5)*	107	63.6	29.0	7.5
HPV negative	5,925	8.0 (7.4-8.8)*	476	86.1	10.9	2.9

Abbreviations: α, α species; N, frequency; ASC, atypical squamous cells of undetermined significance; HSIL+, high-grade squamous intraepithelial lesions or worse.

*Percentage of women with cytologic abnormalities (ASC or worse). Significant findings at the 95% confidence level relative to all other single HPV infections except the HPV type/group in question are indicated by an asterisk.

†Of the women with abnormal cytologic interpretations, the percentage of women with ASC, LSIL, and HSIL or worse were reported.

‡Of the HPV type (single infections), HPV57 had no positive findings.

§Bold indicates the HPV type is one of the 14 HPV types we categorized as carcinogenic.

‖Categorized as a possibly carcinogenic HPV type.

of invasive cancer). Although the percentage of overall cytologic abnormalities were higher in women with multiple infections (40.4%) than observed in women with only single infections (Table 1), the percentage of HSIL and worse cytologic interpretations (21.8%) among women with any cytologic abnormality (ASC or worse) was similar ($P = 0.6$).

Specimen collection. Two exfoliative cervical specimens were obtained during a single pelvic examination at baseline (23). The first specimen was collected using a Cervex brush directed at the cervical os. Following preparation of conventional Papanicolaou smears, thin-layer cytology slides (ThinPrep, Cytyc Corp., Marlborough, MA) from the remaining cells stored in PreservCyt (Cytyc) were made. A second cervical specimen was similarly collected immediately following the first using a Dacron swab and stored in specimen transport medium (STM; Digene Corp., Gaithersburg, MD).

HPV DNA testing. PCR testing was done using DNA extracted from the STM specimen. To amplify HPV DNA, we used a MY09/M11 L1 consensus primer PCR (MY09/11 PCR) method with TaqGold polymerase as described previously (24). In addition, dot blot hybridization of PCR products for HPV type-specific detection was conducted using type-specific oligonucleotide probes for HPV types 2, 6, 11, 13, 16, 18, 26, 31 to 35, 39, 40, 42 to 45, 51 to 59, 61, 62, 64, 66 to 74, 81 to 85, 82 (AE2 and W13B), and 89 (25). Probes for HPV types 2, 13, 34, 42 to 4, 57, 64, 69, 74, 82 (AE2 and W13B), and 54 (AE9) were also combined in dot blot hybridizations for detection of rare types (dot blot mix). Specimens that were HPV positive based on a radiolabeled generic probe mix but were not positive for any type-specific probe were considered to be positive for uncharacterized HPV types.

For these analyses, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (1) plus HPV66 (26) were considered as the primary carcinogenic types. HPV phylogenetic species (in the α genus) that infect the mucosal epithelia were grouped according to our previously published Bayesian phylogenetic tree (27). In addition to individual α species, we also examined five α "species groups." Two groups contain mostly carcinogenic types: (*a*) α9/α11, HPV types 16, 31, 33, 34, 35, 52, 58, 64, 67, and 73, and (*b*) α7, HPV types 18, 39, 45, 59, 68, 70, and 85. One group is a mix of carcinogenic and noncarcinogenic types: (*c*) α5/α6, HPV types 26, 51, 53, 56, 66, 69, and 82. Two other groups contain exclusively noncarcinogenic HPV types: (*d*) α3/α15, HPV types 61, 62, 71, 72, 81, 83, 84, and 89 and (*e*) α1/α8/α10/α13, HPV types 6, 11, 32, 40, 42, 54, 55, and 74.

To determine HPV PCR positivity, three experienced investigators interpreted type-specific dot blot results and discrepancies were resolved by consensus. Signal strength of the PCR products was then evaluated by two observers using a qualitative index originally on a scale of 1 to 5 (weakest = 1 and strongest = 5). The index depicts the strength of the hybridization signal as determined by examining the density and diameter of the PCR product on the autoradiogram (28). PCR signal strength has previously been correlated with the Hybrid Capture assay, a semiquantitative HPV viral load measurement (29), and more recently with the Hybrid Capture 2 assay.[7] Further, examination of the relationship between PCR signal strength and quantitative Taqman PCR, the referent standard of quantitative HPV viral load measurement (30, 31), in women infected with a single type (HPV16 or HPV18) from this population revealed reasonable agreement.[8] We therefore used these measurements as a qualitative measurement of HPV viral load.

Outcome measures. Masked to HPV test results, liquid-based cytology slides were classified with the Bethesda System into normal, ASC, LSIL, HSIL, and cancer by a single reader (M.L.H.). The cytopathologist also made a distinction between HSIL that seemed less severe (CIN2) or more severe (CIN3). Cytologic abnormality was defined as enrollment interpretations of equivocal (ASC) or worse for these analyses. For women with abnormal cytologic interpretations, the percentage of women with equivocal (ASC), mildly abnormal (LSIL), or severe (HSIL) or worse findings were reported. Of the 72 women with both a single HPV infection and a HSIL or worse cytology, 36 women had HSIL-CIN2 cytologic interpretations, 31 women with cytologic interpretations of HSIL-CIN3 interpretations, 4 women with cytologic interpretations of microinvasive cancer, and a single woman had an invasive cancer interpretation.

Statistical analysis. We summarized the frequency and percentage of the type-specific occurrences and used the binomial distribution to calculate the exact 95% confidence intervals (95% CI). We then compared the difference in occurrence of cytologic abnormalities between specific types or species groups using the Pearson χ^2 test. Analyses stratifying these groups by age group (<35, 35-54, and >54 years) were also done. Two-tailed *P*s < 0.05 were considered significant.

We also examined the association of type-specific qualitative viral load with any cytologic abnormality (ASC or worse), LSIL or worse, or HSIL or

[7] J. Palefsky et al. Quantitation of cervicovaginal HPV DNA level and its associations with HPV persistence and incident cervical lesions in HIV-seropositive women, in preparation.

[8] P. Gravitt et al. Viral load of HPV16 is not uniquely associated with prevalent histologic cervical disease but distinctively associated with progression to high-grade neoplasia, in preparation.

worse cytologic abnormalities and for the HPV groups mentioned above. As our patterns for any cytologic abnormality and LSIL or worse did not substantially differ, only results for any cytologic abnormality are shown. For the purposes of type-specific and species analyses, viral load findings were collapsed in a biologically relevant manner [PCR signal strength index of 1 (low) versus 2 to 3 (moderate) versus 4 to 5 (high)]. Alternative groupings did not meaningfully change the conclusions. Assuming a linear relationship for our three-level PCR signal strength variable, we evaluated each HPV type and HPV group using a two-sided test for trend (P_{trend}). For the purposes of our age-species group stratified analyses, PCR signal strength indices of 1 to 3 (lower viral load) were grouped and compared with grouped indices of 4 to 5 (higher viral load). The presence of multiplicative interactions between age group, HPV risk group, and viral load was assessed by use of a Wald χ^2 test with inclusion of the corresponding interaction term of each pair in logistic regression models under the null hypothesis of no difference in risk estimates between groups. We observed no significant interactions.

Results

The order of the presentation of HPV types in Table 1 follows phylogenetic relatedness (27). Among 1,222 women having a single HPV type infection at enrollment, the overall percentage of women

Table 2. Percentage of women with cytologic abnormalities by qualitative viral load

HPV type/group/species		Qualitative viral load (PCR signal strength index)*							
		Low (1)		Moderate (2-3)		High (4-5)		Total N	P_{trend} †
		N	% Abnormal ‡	N	% Abnormal	N	% Abnormal		
All single infections		203	13.3	403	18.1	616	33.0	1,222	**<0.0001**
α10	HPV6	6	16.7	7	0.0	10	30.0	23	0.4
	HPV11	2	50.0	3	0.0	0		5	0.2
	HPV74	0		1	100.0	0		1	
	HPV42	1	0.0	0		2	0.0	3	
	HPV55	0		4	25.0	0		4	
α8	HPV40	2	0.0	2	100.0	0		4	**0.05**
α1	HPV32	4	0.0	3	0.0	3	0.0	10	
α13	HPV54	3	0.0	18	22.2	1	0.0	22	0.6
α9	**HPV52**§	4	0.0	15	20.0	20	20.0	39	0.5
	HPV67	0		3	100.0	2	50.0	5	0.2
	HPV33	2	0.0	6	33.3	15	33.3	23	0.5
	HPV58	9	0.0	16	18.8	45	44.4	70	**0.004**
	HPV16	22	0.0	42	19.1	92	56.5	156	**<0.0001**
	HPV31	4	0.0	18	16.7	24	37.5	46	**0.05**
	HPV35	3	33.3	2	100.0	7	42.9	12	1.0
α11	HPV73‖	3	33.3	1	0.0	5	80.0	9	0.2
	HPV34	1	0.0	0		0		1	
	HPV64	0		1	0.0	0		1	
α7	**HPV59**	2	0.0	5	80.0	2	0.0	9	1.0
	HPV18	10	20.0	10	30.0	19	36.8	39	0.4
	HPV45	3	33.3	2	0.0	11	9.1	16	0.3
	HPV70	18	11.1	27	11.1	40	35.0	85	**0.02**
	HPV39	4	50.0	12	16.7	14	64.3	30	0.2
	HPV68	1	100.0	4	25.0	8	37.5	13	0.5
	HPV85	5	0.0	6	0.0	15	6.7	26	0.4
α5	HPV26‖	1	0.0	2	0.0	2	50.0	5	0.2
	HPV51	16	25.0	21	19.1	35	45.7	72	**0.08**
	HPV82‖	2	0.0	1	0.0	3	33.3	6	0.3
	HPV69	0		0		1	0.0	1	
α6	HPV53	11	36.4	16	12.5	47	19.2	74	0.4
	HPV56	1	0.0	11	27.3	15	66.7	27	**0.03**
	HPV66	1	0.0	9	33.3	8	62.5	18	0.1
α15	HPV71	21	4.8	38	10.5	59	18.6	118	0.09
α3	HPV61	23	8.7	39	7.7	39	0.0	101	0.1
	HPV72	3	0.0	3	0.0	4	25.0	10	0.3
	HPV62	2	50.0	18	11.1	34	17.7	54	0.8
	HPV81	2	50.0	9	11.1	15	26.7	26	1.0
	HPV83	7	14.3	17	23.5	14	0.0	38	0.2
	HPV89	0		4	25.0	0		4	
	HPV84	4	25.0	7	14.3	5	60.0	16	0.2

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Table 2. Percentage of women with cytologic abnormalities by qualitative viral load (Cont'd)

HPV type/group/species	Qualitative viral load (PCR signal strength index)*							
	Low (1)		Moderate (2-3)		High (4-5)		Total N	P_{trend} †
	N	% Abnormal ‡	N	% Abnormal	N	% Abnormal		
α1/α8/α10/α13	18	11.1	38	21.1	16	18.8	72	0.5
α9/α11	48	4.2	104	23.1	210	46.7	362	**<0.0001**
α9/α11 (HPV16-)	26	7.7	62	25.8	118	39.0	206	**0.001**
α7	43	18.6	66	19.7	109	32.1	218	**0.05**
α5/α6	32	25.0	60	20.0	111	37.8	203	**0.04**
α3/α15	62	11.3	135	11.9	170	14.7	367	0.4
All carcinogenic	82	13.4	173	23.7	315	45.7	570	**<0.0001**
Carcinogenic (HPV16−)	60	18.3	131	25.2	223	41.3	414	**<0.0001**
Noncarcinogenic	121	13.2	230	13.9	301	19.6	652	0.06

Abbreviation: Total N, sum of frequencies.

*PCR signal strength was originally a five-level variable collapsed into a three-level variable. The "1" indicates the weakest response; "2 to 3" indicates an intermediate response; and "4 to 5" indicates the strongest response. PCR signal strength for individual HPV types, HPV groups, and species were characterized using HPV type-specific PCR probes.

†P_{trend} indicates a two-sided trend test; bold *P*s suggest significance at the 95% confidence level.

‡Abnormal includes women with atypical squamous cells of undetermined significance or worse cytologies.

§Bold indicates the HPV type is one of the 14 HPV types we categorized as carcinogenic.

‖Categorized as a possibly carcinogenic HPV type.

with cytologic abnormality (ASC or worse) were 24.8% (95% CI, 22.4-27.3; Table 1). Importantly, we found that 25.7% (α7) to 45.5% (α11) of women with mainly carcinogenic species (α9, α11, α7, α5, and α6) had concurrent cytologic abnormalities considerably more than women with noncarcinogenic HPV types in aggregate (16.4%; 95% CI, 13.6-19.5; Table 1). In fact, women with single α3/α15 noncarcinogenic HPV type infections were only slightly more likely to have cytologic abnormalities than HPV DNA-negative women, although the difference was statistically significant because of large numbers yielding very small confidence intervals (13.1% versus 8.0%, respectively; $P = 0.0007$).

Interestingly, 38.5% (95% CI, 30.8-46.6) of women with HPV16, the most common HPV type of the α9 species, had cytologic abnormalities. This percentage of cytologic abnormalities was near the middle of the range for individual α9 types [range, 18.0% (HPV52) to 80.0% (HPV67)] and for other individual carcinogenic types [range, 12.5% (HPV45) to 50.0% (HPV35)]. The percentage of cytologic abnormalities associated with HPV16 infection were also similar to percentage of women with any other of the carcinogenic types in aggregate (32.9%; 95% CI, 28.3-37.6; $P = 0.2$).

Thirty of 40 individual HPV types examined (75.0%), including HPV16, produced as much or more equivocal (ASC) as definite (LSIL) viral cytopathic effect (Table 1). Only four HPV types (10.0% of all HPV types; HPV16, HPV31, HPV26, and HPV85) had more concurrent HSIL or worse cytologies than ASC or LSIL cytologies combined. Of note, HPV26 ($n = 5$) and HPV85 ($n = 26$) were rarely detected and only one woman for each type had an abnormal cytology interpreted as HSIL or worse. HPV16-positive women had the greatest percentage of abnormalities interpreted as HSIL or worse (60.0%).

Therefore, the two major cancer-associated HPV species groups (α9/α11 and α7) differed considerably with regard to typical cytologic severity when abnormalities were detected (Table 1). Among women with α9/α11 type-associated abnormalities, 29.0% were interpreted as ASC, 26.6% were interpreted as LSIL, and 44.4% were interpreted as HSIL or worse. Women with α7 types (HPV18, HPV45, and related types), in contrast, had a strikingly low percentage of HSIL or worse (10.7%), which was significantly less than the α9/α11 species group ($P = 0.03$). When abnormal cytology was (uncommonly) observed for women with α3/α15 HPV types, most were interpreted as ASC (68.8%), 25.0% were interpreted as LSIL, and very few (6.3%; $n = 3$) were interpreted as HSIL or worse.

Phylogenetic relatedness did not completely explain the variability within species and species groups. For example, although HPV53, HPV56, and HPV66 are members of the α6 species, HPV53 showed significantly less overall cytologic abnormalities (20.3%; 95% CI, 11.8-31.2; $P = 0.002$) than the carcinogenic HPV56 and HPV66 types together (46.7%; 95% CI, 31.7-62.1) and there were notably different percentages of ASC, LSIL, and HSIL or worse interpretations.

Exploring the relationship between cytologic abnormality and viral load, we found that increasing qualitative viral load (as measured by PCR signal strength) of any single HPV type was significantly associated with cytologic abnormalities ($P_{trend} < 0.0001$; Table 2), an effect that was largely driven by α9/α11 HPV types (in aggregate, $P_{trend} < 0.0001$) and, more specifically, HPV16 ($P_{trend} < 0.0001$). Although 8 of 42 (19.1%) women with moderate HPV16 qualitative viral load and 52 of 92 (56.5%) women with higher HPV16 qualitative viral loads had cytologic abnormalities, none of the 22 women with low HPV16 qualitative viral load were interpreted as abnormal (Table 2). The association between greater qualitative viral load and abnormal cytology was significant for α5/α6 types ($P_{trend} = 0.04$) and marginally significant for α7 types ($P_{trend} = 0.05$), the other α species that contain carcinogenic HPV types. However, no significant trends were observed for noncarcinogenic α1/α8/α10/α13 types ($P_{trend} = 0.5$) and α3/α15

types (P_{trend} = 0.4). Examination of all noncarcinogenic types in aggregate (including those in α9/α11/α7/α5/α6 species) revealed a weak, nonsignificant linear trend (P_{trend} = 0.06).

Because most cytologic abnormalities are the result of HPV infection and because we previously observed a U-shaped age-prevalence pattern for HPV DNA positivity (16), we examined whether there was a similar pattern for cytologic abnormalities among all women in the study and among HPV-positive women. Instead of a U-shaped curve, we observed a substantial reduction in abnormalities in the older age group compared with either of the younger age groups. For the overall study population, including HPV-negative women, the percentage of abnormalities for the three age groups were 11.8%, 12.6%, and 5.4%, respectively. We observed a similarly reduced percentage of cytologic abnormality among older women when we examined only HPV-positive women as shown in Table 3 (P < 0.0001).

Although only 22.7% of older women with HPV16 infection had cytologic abnormalities, four of these five women had HSIL or worse interpretations. Similarly, 50.0% of the women with cytologic abnormalities due to other α9/α11 types in the older age group had HSIL or worse interpretations, whereas none of the cytologically abnormal women infected with exclusively noncarcinogenic species groups (α1/α8/α10/α13 and α3/α15) had HSIL or worse interpretations (Table 3). Further, we observed that the lowest percentages of abnormalities predominantly occurred among the oldest age group regardless of viral load or species group (Fig. 1). However, the trends were not simple or monotonic. For some species groups, but not others, cytologic abnormalities increased in the middle age group before falling at older ages.

Discussion

In this analysis, we investigated associations of cytologic abnormality with type-specific HPV infection, qualitative viral load based on type-specific PCR signal strength, and age. We tested for the full range of anogenital HPV types in a large population sample of women with single HPV infections. Our analyses revealed that (*a*) the percentage of cytologic abnormality varied greatly depending on the infecting HPV type, with types within carcinogenic species producing similar higher levels of cytologic abnormalities; (*b*) for these same high-risk profile groupings, cytologic abnormality was significantly associated with high qualitative viral loads, with weaker associations among older women and no such associations for noncarcinogenic species; (*c*) percentages of cytologic abnormality for most types declined among older women (>54 years); (*d*) HPV16-positive women did not have more overall cytologic abnormalities in our study compared with women with other carcinogenic types, but HSIL or worse findings were more commonly found in these women compared with other types, particularly among older women; and (*e*) in contrast to HPV16-related types, HPV18 and the related α7 species types overall were associated with strikingly lower percentages of HSIL or worse cytologic interpretations.

HPV type, viral load, and abnormality. Previous reports have indicated that the risk of HPV persistence and disease progression differ greatly by HPV type with genetically related types appearing to behave most similarly (2, 27). These analyses show that the percentage of cytologic abnormality varies by HPV type, and further, we have shown that three species groups (α5/α6, α7, and α9/α11) have nearly equivalent proportions of cytologic abnormality (~30%; Table 1). These species groups consist primarily of carcinogenic and possibly carcinogenic HPV types. The remaining species groups that include only noncarcinogenic types are only half as likely to produce cytologic abnormalities. In our study, genetic relatedness did not completely explain differences in cytologic abnormalities observed within species; there are likely to be still unknown biological properties that distinguish individual viral types. These properties might directly influence the ability of the viruses to replicate or reflect differences in properties of viral gene expression.

Previous reports have suggested that high HPV DNA copy number is associated with cytologic abnormalities (32) and that HPV-positive women with normal cytology are often observed to have very low viral loads with minimal risk of subsequent progression to cancer (9, 17). Using PCR signal strength as a qualitative measure of viral load, we observed that the single strongest significant positive linear relationship between viral load and cytologic abnormality was found in HPV16-positive women (P_{trend} < 0.0001). Cytologic abnormality was correlated to a lesser extent with high HPV viral load in women with other α9/α11 (primarily carcinogenic) HPV types (Table 2). As specimens for cytology and HPV testing were similarly collected, it is likely that viral load and cytologic abnormality are measuring the same phenomenon and, further, that biological or genetic properties of specific virus types modulate these two highly correlated outcomes.

The influence of age on abnormality. Our earlier analyses of the Guanacaste cohort reported an early decline in HPV prevalence with age followed by a second albeit lower peak in prevalence after menopause (16). If HPV positivity is driving abnormality in the same way in women of all ages, we would expect nearly equivalent percentages of cytologic abnormalities in HPV-positive women across all three age groups. In contrast, our present data show that the proportions of singly infected women exhibiting cytologic abnormalities were analogous to women in the <35 and 35- to 54-year-old age groups but significantly lower in the >54-year-old age group (Table 3). Viral load was not consistently related to age for all women or stratified by level of cytologic abnormality.

Numerous mechanisms may simultaneously contribute to the complex observation of decreased likelihood of cytologic abnormalities in older women. For example, changing hormone levels in aging women results in atrophy (thinning of the cervix) and replacement of the squamocolumnar epithelium by vaginal squamous epithelium. These events may result in the collection of cells less predisposed to HPV-induced cytopathologic changes, consistent with reports that fewer cytologic abnormalities are observed in hysterectomized compared with nonhysterectomized women (33).[9]

In addition, tropism for the vaginal epithelium rather than the cervical epithelium by the more prevalent noncarcinogenic HPV types might decrease detection of cytologic abnormalities. Indeed, we previously found that noncarcinogenic α3/α15 types have a predilection for vaginal epithelium (34). Our present data indicated that 39.9% of the age group of >54 years had α3/α15 types and that only 6.3% of these women had cytologic abnormalities.

Interestingly, we observed a striking absence of LSIL in the >54 year-old age group (Table 3). We cannot fully explain this observation. Due to atrophy of the epithelium, HPV infections in older women may only produce very subtle cytologic changes with fewer and smaller koilocytes. Microscopic detection of these often-transient koilocytotic changes (categorized as LSIL) might

[9] P.E. Castle et al. Human papillomavirus (HPV) prevalence in hysterectomized and nonhysterectomized women. J Infect Dis. In press 2006.

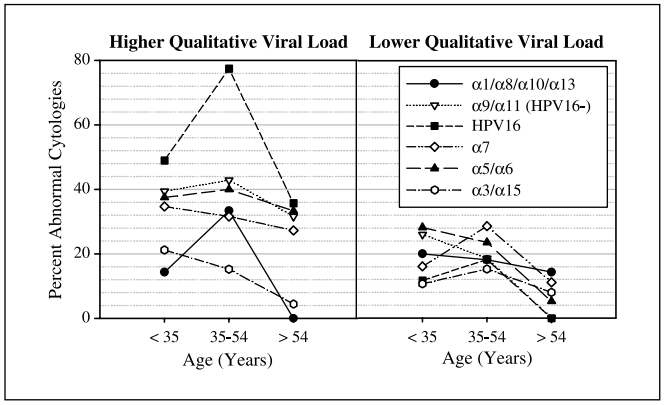

Figure 1. Association of cytologic abnormality with age by α species and qualitative HPV viral load. Abnormal cytologies are defined as ASCs or worse cytologic interpretations. Lower viral load indicates PCR signal strength indices of 1 to 3. Higher viral load indicates PCR signal strength indices of 4 to 5.

consequently be more difficult. More studies, however, are needed to completely understand the influence of age on the natural history and biological effects of HPV infection.

Study strengths and limitations. Use of PCR signal strength in this study allowed a first examination of viral load for the full range of anogenital HPV types in a true population-based cohort. Other studies primarily have focused on disease associations with HPV16 infection (9, 35–39) and a handful other individual HPV types (generally no more than 10 types in a single study; refs. 31, 40–43). Nevertheless, PCR signal strength is a relative estimator of viral load and not a quantitative measure. As such, we were not able to control for cellularity in our analyses. However, any measure of viral load using exfoliated cells is in reality qualitative because it is impossible to differentiate 1,000 viral copies in one cell from 1,000 cells containing a single viral genome each. In addition, similar to other viral load measures, we were not able to account for lesion size. Previous reports have suggested that lesion size, in addition to lesion severity, may influence viral load measurements (11, 44). Also important, we were unable to examine viral load within women with multiple infections (~35% of HPV infections in this study population), limiting the generalizability of our data to women with single HPV infections. Last, it is possible that we have underestimated associations between HPV type, viral load, and cytology given the sequential collection of specimens. Further studies and/or pooled analyses are therefore needed to corroborate and extend our findings.

Clinical implications. The major clinical implications of our findings relate to HPV16 and HPV18, the major carcinogenic types worldwide. HPV16 is the type most likely to cause cytologic abnormalities, which, when present, tend to be HSIL or worse (especially among older women if the estimates based on small numbers prove correct). In contrast, HPV18 is unlikely to cause HSIL or worse cytologies despite its importance in causing 37% to 41% of cases of cervical adenocarcinoma (which in turn represents ≥15% of all cervical cancers; ref. 45). The qualitative difference in cytopathic effect, as seen in an unbiased population study of adult women from age 18 to 97, is remarkable and supports earlier data from case series (46) and prospective data (27, 47). No one has been able to explain exactly why HPV18 tends

Table 3. Percentage of women with cytologic abnormalities stratified by age

Type/group	N_{Total}*	Overall % abnormal cytologies	N_{ABN+}†	% Abnormal within age group (y)‡			N_{ASC}§	% ASC within age group (y)‖			N_{LSIL}	% LSIL within age group (y)			N_{HSIL+}	% HSIL or worse within age group (y)		
				<35	35-54	>54		<35	35-54	>54		<35	35-54	>54		<35	35-54	>54
All single infections	1,222	24.8	303	26.8	28.3	13.9	134	43.1	46.2	42.4	97	37.9	26.5	24.2	72	19.0	27.4	33.3
α1/α8/α10/α13	72	18.1	13	18.5	21.4	11.8	9	80.0	50.0	100.0	4	20.0	50.0	0.0	0			
α9/α11	362	34.3	124	33.7	41.7	21.2	36	30.9	26.7	27.3	33	32.4	22.2	9.1	55	36.8	51.1	63.6
α9/α11 (HPV16−)	206	31.1	64	33.9	30.9	20.0	23	34.2	41.2	33.3	22	39.0	29.4	16.7	19	26.8	29.4	50.0
α7	218	25.7	56	24.8	30.1	20.0	29	50.0	54.6	50.0	21	46.2	31.8	25.0	6	3.9	13.6	25.0
α5/α6	203	30.5	62	33.7	32.4	17.7	27	40.6	50.0	33.3	27	53.1	33.3	33.3	8	6.3	16.7	33.3
α3/α15	367	13.1	48	15.6	15.3	6.3	33	68.2	75.0	50.0	12	27.3	15.0	50.0	3	4.6	10.0	0.0
All carcinogenic	570	34.4	196	34.8	39.6	21.0	66	34.3	35.1	23.5	66	40.0	27.0	23.5	64	25.7	37.8	52.9
HPV16	156	38.5	60	33.3	52.8	22.7	13	25.9	17.9	20.0	11	22.2	17.9	0.0	36	51.9	64.3	80.0
Carcinogenic (HPV16−)	414	32.9	136	35.3	34.3	20.3	53	37.2	45.7	25.0	55	46.2	32.6	33.3	28	16.7	21.7	41.7
Noncarcinogenic	652	16.4	107	17.9	18.9	10.2	68	62.5	65.1	62.5	31	33.3	25.6	25.0	8	4.2	9.3	12.5

Abbreviation: ABN+, abnormal cytologies; ASC, atypical squamous cells; LSIL, low-grade intraepithelial lesions; HSIL+, high-grade intraepithelial lesions or worse.

*Total frequency of women.

†Frequency of women with cytologic abnormalities or ASC or worse cytologies.

‡Percentage of women within each age group with abnormal or ASC or worse cytologies. There were 153, 117, and 33 abnormal women in the <35, 35–54, and >54 year-old age groups, respectively.

§Frequency of women with ASC cytologies.

‖Percentage of similarly aged women with cytologic abnormalities that have ASC cytologies.

to be "occult" at the stage of high-grade intraepithelial lesions (precancer), the target of cervical cancer screening. Differences in viral activity could be involved or it could be a correlate of the typical cell target (i.e., the relative lack of exfoliation during screening of endocervical glandular cells simulating lower viral load and fewer abnormal cells). Regardless, in screening we can expect HPV16 infections to reveal themselves more aggressively compared with HPV18 infections. In a parallel, prospective study in the same Guanacaste cohort, HPV18 accounted for four and HPV45 accounted for another one of the nine invasive cancers that occurred despite vigorous screening (27). Our data suggest that we should pay careful attention to HPV18 as well as to HPV16 but for a different reason. Specifically, the possible use of HPV18 typing to improve the detection of cytologically occult lesions should be formally evaluated.

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